Antioxidant Activity Of Nanoemulsion Of Beet Fruit Extract Using The Dpph Method

Ahmad Azrul Zuniarto, Siti Pandanwangi, Subagja, Ris Ayu Nuari, Desak Gde Listya Paramitha, Farhatun Nisa *)
Faculty of Pharmacy, Imam Bonjol Majalengka Education Foundation University
*email: farhatun0404@gmail.com

Abstract

Free radicals are one of many forms of reactive oxygen sorts known for having unpaired electrons. The presence of free radicals in the body can cause damage such as premature aging, characterized by the appearance of wrinkles. This damage can be countered with antioxidant compounds. Beetroot is one of the fruits with antioxidant properties due to the presence of betacyanin compounds from the flavonoid group. This research aims to identify the antioxidant activity and IC50 value of beetroot (Beta vulgaris L.) extract nanoemulsion formulations using the DPPH method. Nanoemulsion formulations were prepared with varying concentrations of beetroot extract at 1% (X1), 3% (X2), and 5% (X3) to determine which concentration has the best antioxidant activity. Quality evaluation of the nanoemulsion formulations was also conducted, including organoleptic tests, pH tests, and homogeneity tests. The results showed that the beetroot extract nanoemulsion formulations have antioxidant activity and meet the requirements for physical properties and formulation stability. Nanoemulsion formulations with 5% (X3) beetroot extract content exhibited the best antioxidant activity with an IC50 value of 136.475 ppm.

Keywords: beta vulgaris L., free radicals, ic50, uv-vis spectrophotometer.

1. INTRODUCTION

One of the symptoms of premature aging is the appearance of wrinkles on the skin which is caused by a decrease in the amount of collagen and elastin in the dermis layer as well as changes in texture in the epidermis layer of the skin. The presence of free radical compounds is a factor that can cause this. Compounds with unpaired electrons or free radicals are a type of reactive oxygen compound. Electrons that don't have a partner are constantly looking for new electron pairs, so they easily react with other substances such as proteins, fats and DNA in the body (Baitariza *et al.*, 2014; Sayuti & Yenrina, 2015).

As a response to gamma radiation, ultraviolet (UV) light, pollution in the environment consisting of vehicle fumes, dirt, garbage fumes, and combustion fumes as well as pollution from household and factory waste disposal, then cigarette smoke, X-rays, and chemical compounds in food (synthetic preservative). Substances that provide color, residual pest control

chemicals, and other food additives) can also induce the continuous formation of free radicals in the body. This process may occur through normal cell metabolism, lack of nutrition, inflammation, antioxidant compounds found in free radicals can reduce the damage they cause. Compounds that are able to inhibit, suspend, or stop the lipid oxidation process are known as antioxidants (Baitariza *et al.*, 2014; Sayuti & Yenrina, 2015).Specifically, an antioxidant is a substance that has the ability to slow down or block the emergence of free radical oxidation reactions in lipid oxidation. Antioxidants play a role in providing or donating one electron to compounds that have oxidant characteristics, which can result in inhibiting the activity of the oxidant compound. The presence of antioxidant compounds can end the process of cell damage by channeling electrons to free radicals. In this way, antioxidants can neutralize free radicals, as a result these free radicals are unable to take any more electrons from cells and DNA (Sayuti & Yenrina, 2015).

Red beet *(Beta vulgaris L)* is a plant from the *Chenopodiaceae family,* which has a tuber-like morphology and is generally used as a vegetable. The distinctive characteristics of red beets are the deep red color of the beet roots, the sweet taste like sugar, and the aroma of the beets which is known as an earthy *taste* (Widyaningrum and Suhartiningsih, 2014).

Beetroot contains betacyanin compounds which are included in the flavonoid group. Based on previous research, people with low blood pressure are advised to consume large amounts of beets because beets contain vitamins and minerals. Based on this, this research took samples of beet plants because they have many health benefits.

Nanoemulsion is a hermodynamically stable lipid-based drug delivery system consisting of oil, surfactant, cosurfactant, and water which has a droplet size of nanometers. The nanoemulsion delivery system is able to increase absorption, solubility of lipophilic drugs and bioavailability. The large surface of the nanoemulsion system will increase the surface area and free energy, so that the delivery system becomes effective because the amount of energy required is less and is thermodynamically stable (Aprilya et al., 2021).

The DPPH method was used to measure the antioxidant activity of beetroot extract nanoemulsion to obtain the IC50 value. DPPH (2,2-diphenyl-1-picrylhydrazil) is a reagent that acts as an oxidizer when it reacts with antioxidant compounds in a sample (Putri MKN, *et al.* , 2015). The DPPH molecule is a free radical that is stable, has a dark purple color, and shows a close absorbance of around 517 nm. DPPH has chromophoric and auxochromic groups.

In previous research, tests were carried out on the physicochemical properties of betacyanin found in red beet tubers (Beta vulgaris L.) and to assess the potential antioxidant activity found in red beet tubers (Beta vulgaris L.) (Asra, R., et *al.,* 2020). This test uses samples of beetroot in extract form and not in dosage form. Meanwhile, in this research, tests were carried out to identify the antioxidant activity of the beetroot extract nanoemulsion using the DPPH test and to determine the concentration of the beetroot extract nanoemulsion preparation which had the best antioxidant activity. Nanoemulsion preparations are made with a

content of 1%, 3%, or 5% beetroot extract (Anindhita & Oktaviani, 2016).

2. METHOD

Types of research

This research uses descriptive analytical research methods.

Place and time of research

The research was carried out from September 2022 to March 2023 at the YPIB Majalengka University campus laboratory.

Tools and materials

The instruments used in this research were a rotary vacuum evaporator (IKA), homogenizer (IKA), UV-Vis spectrophotometer (BIOBASE), pH meter (HANNA), thermometer (PYREX), magnetic stirrer (DLAB), sonicator (SXSONIC), spatula , pipette, Erlenmeyer flask (IWAKI), beaker (IWAKI), measuring cup (IWAKI), glass bottle, test tube (IWAKI), electronic measuring device (FUJITSU), knife, cutting mat, and filter paper.

The ingredients used in this research include beetroot, 70% ethanol, Tween 80, Span 80, pharmaceutical quality propylene glycol obtained from PT. Dipa Prasada Husada, while VCO, vitamin C powder, and DPPH powder (1,1-diphenyl-2-picrylhydrazil) with pro-analyst quality were obtained from Merck. And aquadest is obtained from Aqua Bidest.

Samples of beetroot obtained from the Jagasatru market, Pekalipan District, Cirebon City. Of the selected beets, 2 kg.

Preparation of Beet Fruit Simplisia (Beta vulgaris L.)

Samples of beetroot (*Beta vulgaris* L.) will be sorted first before being washed with running water. Once clean, the beets are then peeled and the flesh is chopped to make the drying process easier in the sun. The dried beetroot simplicia is then ground with a blender until it becomes powder and then stored in a tightly closed container.

Preparation of Beetroot Extract (Beta vulgaris L.)

A total of 143 grams of beetroot powder was put into a tight container (macerator) and 1,072 mL of 70% ethanol solvent was added. This maceration process was carried out for 7 days at room temperature and protected from exposure to the sun, with the macerator covered with aluminum foil. While the simplicia is soaking, stir occasionally. After 7 days, the macerate is then filtered using a white cloth placed on a beaker glass. The results are then evaporated using *a rotary evaporator* to obtain a thick extract.

Phytochemical Screening (Flavonoid Identification)

0.5 g of sample was dissolved in 50 mL of water and then heated for about 5 minutes. The sample was then filtered and 5 mL of the result was taken and then mixed with 1 mg of Mg powder in a test tube. Next, add 1 mL of chlorhydric alcohol solution (a combination of 37% HCl and 95% ethanol in equal volumes) and a few drops of amyl alcohol, stir vigorously, and allow separation to occur. The appearance of color in amyl alcohol (red, yellow or orange) indicates the presence of flavonoid compounds.

Preparation of Beet Fruit Nanoemulsion (*Beta vulgaris* L.)

The beetroot extract filtrate was dissolved in span 80, which was then mixed with VCO (oil phase) using a *magnetic*

stirrer for around 30 minutes at a temperature of 37º C with a speed of 1000 rpm until it reached a homogeneous condition. Tween 80 is mixed with propylene glycol and propyl paraben then homogenized using a homogenizer at a temperature of 37º C at a speed of 1000 rpm, this combination is referred to as the water phase. Next, the oil phase was introduced into the water phase gradually, and homogenized for 30 minutes at a temperature of 37º C at a speed of 1000 rpm. The preparation was then placed in a Sonicator for 15 minutes at a temperature of 37º C until a nanoemulsion was formed.

Quality Evaluation of Beetroot Extract Nanoemulsion (*Beta vulgaris* L.)

1. Organoleptic Test

This evaluation aims to detect the color, shape and aroma of the nanoemulsion preparation during the storage period. This procedure involves the sense of smell to detect the odor of the preparation and make visual observations of the color and shape. Clear, odorless and liquid preparations are considered good organoleptic substances.

2. Test pH

pH testing for each formula is carried out using a pH meter. 10 ml of the nanoemulsion preparation was taken, then an electrode was inserted into it to record the pH value which can be seen on the pH meter. The pH value of a good preparation is 6.5-9.0.

3. Homogeneity Test

Observation of whether the formulation is homogeneous or not is carried out by evaluating the presence or absence of lumps or coarse particles in the formulation.

Antioxidant Activity Test of Beet Fruit Extract Nanoemulsion (*Beta vulgaris* L.)

After the absorbance value appears, it is then entered into the % inhibition formula to identify antioxidant activity or free radical scavengers using the formula:

$$\% \textbf{ Inhibisi } = \frac{\mathbf{a - b\ X\ 100\%}}{\mathbf{a}}$$

Information:

- a = Blank absorbance
- b = Sample absorbance

Then, enter it into the linear regression equation to calculate the IC $_{50\ value}$ using the formula:

$$\mathbf{Y = bx + a}$$

Determination of IC $_{50}$ (*Inhibiton Concentration*) Value

The amount of antioxidant activity is indicated by the IC50 value, namely the concentration of the sample solution needed to inhibit 50% of DPPH free radicals.

Plots of sample concentration and percentage inhibition are arranged on the X-axis and Y-axis of the linear regression equation. This equation is used to identify the IC $_{50\ value}$ of each sample, which is determined when the Y value reaches 50, and the X value corresponding to IC $_{50}$ can be obtained.

3. RESULTS AND DISCUSSION

Extraction

The results of the extraction process carried out using the maceration method were that beetroot simplicia experienced a shrinkage of 92.5% and then 97.51% of the

thick extract was extracted from 143 grams of beetroot simplicia. The result was 65% beetroot extract.

Phytochemical Screening

Identification of flavonoid compounds using alcohol and chloral hydrate reagents obtained positive results which were indicated by the appearance of color in the amyl alcohol (yellow, orange or red).

After carrying out phytochemical tests, flavonoid compounds were found in beetroot extract. The formation of a red color or hue in amyl alcohol is a sign of positive results. To detect flavonoids, magnesium powder and concentrated HCl must be added to break the bond between glycosidic and flavonoids.

Betacyanin is a red and violet dye which belongs to the flavonoid group which has polar properties because it binds to sugar, and is a nitrogenous pigment which is a substitute for anthocyanin (Novatama, 2016). In red botany, Betacyanin exhibits a number of high antioxidant activities as well as anti-free radical effects.

Preparation of Beetroot Extract Nanoemulsion (*Beta vulgaris* L.)

Beetroot extract (*Beta vulgaris* L.) nanoemulsion was made with three different concentrations, namely 1%, 3% and 5%. Weighing of preparations can be seen in Table 1.

Table 1. Results of weighing nanoemulsion ingredients for beetroot extract (*Beta vulgaris* L.)

No	Ingredients	Formulation (g)			
		X 1	X 2	X 3	K-
1	Beetroot Extract (Beta vulgaris L.)	1 gram	3 grams	5 grams	-
2	VCO	1.5 grams	1.5 grams	1.5 grams	1.5 grams
3	Propylen glycol	2.5 grams	2.5 grams	2.5 grams	2.5 grams
4	Teen 80s	13.5 grams	13.5 grams	13.5 grams	13.5 grams
5	Span 80	0.5 grams	0.5 grams	0.5 grams	0.5 grams
6	Propyl paraben	0.3 grams	0.3 grams	0.3 grams	0.3 grams
7	Aquades	add 100	add 100	add 100	Add 100

According to Anindhita & Oktaviani (2016), nanoemulsion formulations were chosen because they can increase bioavailability, solubility, dissolution rate, and absorption of active compounds in the body. This preparation is made with distilled water as the water phase, Tween 80 and Span 80 as the surfactant, propylene glycol as the consurfactant, propyl paraben as the humectant, and pure coconut oil (VCO) as the oil phase.

Evaluation Results of Beet Fruit Extract Nanoemulsion Preparations

The evaluation results which include organoleptic observations and pH tests of the nanoemulsion preparation can be seen in Table 2.

Table 2. Quality Evaluation Results of Beetroot Extract Nanoemulsion (Beta vulgaris L.)

Formulas	Quality Evaluation			
	Organoleptic			pH
	Form	Smell	Color	
X 1	Liquid	Sweet	Light Maroon	6.9
X 2	Liquid	Sweet	Maroon	6.7
X 3	Liquid	Sweet	Deep Maroon	6.6
K-	Liquid	Typical VCO	Cloudy White	7.0

On organoleptic observation, the preparation of Red Beet Extract Nanoemulsion (*Beta vulgaris* L.) is liquid, red in color, and has a distinctive sweet smell. Differences in the concentration of beetroot extract were proven to influence the preparation of nanoemulsions as seen in Figure 1.

Figure 1 . Formulation Results of 1%, 3%, 5%, and K - beetroot extract nanoemulsion preparations

The higher the concentration of beetroot extract added, the more intense the red color in the preparation will be. Meanwhile, the nanoemulsion base is liquid, has a distinctive smell of VCO, and is cloudy white in color.

From the three preparation formulations tested, pH data was obtained. The higher the concentration, the more acidic the pH of the preparation. This occurs because extracts from natural ingredients usually have acidic properties so that increasing the concentration of the extract will result in a decrease in pH. Meanwhile, the nanoemulsion base as a negative control has a pH of 7 or neutral. The pH results obtained are in accordance with the pH test parameters, namely 6.5-9.0.

The homogeneity test of the preparation is carried out to see whether the globules of the dispersed substance are evenly distributed with various sizes in the disperser. Based on the results of the homogeneity test that has been carried out, all preparation formulas are homogeneous. This homogeneity indicates the stability of the nanoemulsion globules due to the appropriate formulation.

Antioxidant Activity Test of Nanoemulsion Preparation of Beet Fruit Extract

Before measuring samples and vitamin C activity, the maximum wavelength is first determined. To achieve accurate and linear measurements, it is recommended to select the wavelength at which the compound being measured has the greatest absorption. At 517 nm, the longest absorption wave can produce a dark purple color because DPPH has chromophore and auxochrome groups.

The IC50 value calculated using the linear regression equation for vitamin C is 6,961 ppm, vitamin C is considered very strong (IC50 50 ppm). Next, antioxidant activity tests were carried out on beetroot extract (*Beta vulgaris* L.) nanoemulsions with a concentration of 1% (X1), a concentration of 3% (X2), and a concentration of 5% (X3) which were created in a concentration series of 20 ppm, 40 ppm, 60 ppm , 80 ppm, and 100 ppm. From each concentration series, 2 mL was taken, then 2 mL of DPPH was added, then homogenized and incubated for approximately 30 minutes in the dark. Then the absorption was measured using UV-Vis spectrophotometry at the maximum wavelength. Based on the results of the linear regression equation, nanoemulsion of beetroot extract (*Beta vulgaris* L.) with a concentration of 1% (X1), concentration of 3% (X2), and concentration of 5% (X3) has a continuous IC50 value of 144,496 ppm, 139,072 ppm, and 136,475 ppm.

It can be concluded that beetroot extract nanoemulsion with a concentration of 1% (X1) has the smallest IC50 value

compared to beetroot extract nanoemulsion with a concentration of 5% (X3) which has the greatest antioxidant activity with an IC50 value of 136,475 ppm in the medium antioxidant category. A compound has a very strong antioxidant if it has an IC50 value of <50 ppm, it is categorized as strong if the IC50 value is 50-100 ppm, in the medium category if the IC50 value is between 100-150 ppm, and in the weak category if the IC50 value is around >150 ppm (Sepriyani *et al. ,* 2020).

The antioxidant activity test results obtained from the nanoemulsion preparation of beetroot extract were not much different from previous research by Sawiji & La (2022), where the antioxidant activity of *the body butter preparation* of ethanol extract of beetroot (*Beta vulgaris* L.) had an IC50 value of 133.34 ppm. These two results show differences with other studies that carried out antioxidant testing on pure extracts of beetroot which were not in dosage form, such as in research by Septiani (2020), where beetroot showed very strong antioxidant activity, namely 7.77 ppm. Other studies also showed similar results where the antioxidant activity of beetroot extract in Asra *et al.*, (2020), has an IC50 value of 21.8878 ppm which is also included in the very strong category.

In addition, the nanoemulsion base was used as a negative control or comparison for the antioxidant activity test. The linear regression equation used for alkaline nanoemulsion (K-) determines that the IC50 is 249,001 ppm, classified as weak category (ICmin >150 ppm).

The extract concentration in this study was related to antioxidant activity, meaning that the concentration or dose of the extract increased its antioxidant effectiveness.

4. CONCLUSION

In the nanoemulsion preparation of beetroot extract, there is antioxidant activity with the best formulation containing 5% beetroot extract which has an $IC_{50\ value}$ of 136.475 ppm.

5. ACKNOWLEDGEMENTS

The author expresses his gratitude to the rectorate of YPIB Majalengka University for granting research to us.

6. BIBLIOGRAPHY

Anindhita, M. A., Oktaviani, N. (2016). Formulasi Self-nanoemulsifying Drug Delivery System (SNEDDS) Ekstrak daun Papaya (Carica papaya L.) dengan Virgin Coconut Oil (VCO) sebagai Minyak Pembawa. Jurnal Pena Medika. 6(2): 103-111.

Aprilya, A., Rahmadevi, R., & Meirista, I. (2021). Formulasi Nanoemulsi dengan Bahan Dasar Minyak Ikan (Oleum Iecoris Aselli). Jurnal Sains dan Kesehatan, 3(3), 9-16.

Asra, R., Yetti, R. D., Ratnasari, D., & Nessa. (2020). Physicochemical Study of Betasianin and Antioxidant Activities of Red Beet Tubers (Beta vulgaris L.). Journal of Pharmaceutical and Sciences. 3(1), 14–21.

Baitariza, A., Darijanto, S. T., Pamudji, J. S., & Fidrianny, I. (2014). Formulasi dan Evaluasi Mikroemulsi Antikerut Ekstrak Beras Hitam (Oryza sativa L.). Ijpst. 1(1): 3–8.

Novatama, & Mutiara, S. (2016). Identifikasi Betasianin Dan Uji Antioksidan Ekstrak Buah Bit

Merah. Jurnal Ilmu Kimia. 5(3): 3–6.

Sawiji, R. T. & La, E. O. J. (2022). Formulasi Dan Uji Aktivitas Antioksidan Sediaan Body Butter Ekstrak Etanol Umbi Bit (Beta vulgaris L.) dengan Metode DPPH. Jurnal Ilmiah Manuntung: Sains Farmasi dan Kesehatan. 8 (1): 173-180.

Tristantini D, Ismawati A, Pradana BT, & Jonathan JG. (2016). Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (Mimusops elengi L). Paper presented at Seminar Nasional Teknik Kimia Kejuangan, Yogyakarta, Indonesia.

Sayuti, K., & Yenrina, R. Antioksidan Alami dan Sintetik. (2015). Andalas Univesity Press: Padang.

Sepriyani, H., Devitria, R., Surya, A., & Sari, S. (2020). Aktivitas Antioksidan Ekstrak Metanol Daun Pepaya (Carica papaya L.) Dengan Metode DPPH. Jurnal Penelitian Farmasi Indonesia, 9(1), DPPH. 10, 863-867

Septiani. (2020). Uji Aktivitas Antioksidan Ekstrak Buah Senggani (Melastoma malabathricum L.) dan Ekstrak Buah Bit (Beta vulgaris L.). KATALIS Jurnal Penelitian Kimia dan Pendidikan Kimia. 3 (2): 35-41.

Putri, M.K.N., Gunawan, I. W. G., & Suarsa, I. W. (2015). Aktivitas Antioksidan Antosianin Dalam Ekstrak Etanol Kulit Buah Naga Super Merah (Hylocereus costaricensis) dan Analisis Kadar Totalnya. J. Kimia. 9(2): 243-251.

Widyaningrum, M. L. & Suhartiningsih. 2014. Pengaruh Penambahan Puree Bit (Beta vulgaris) terhadap Sifat Organoleptik Kerupuk. Jurnal Boga. 3(1) 233-238.